

plasmid (a gift from Dr. J. Sodroski, Dana-Farber Cancer Institute, Boston, MA). One fragment was generated by PCR with the synthetic oligonucleotide containing the *Sa*II site and the CCACC Kozak's sequence in front of the ATG codon (5'-AGAGTCGACCCACCATGAGAGTGAAGGAGA-3', sense) (SEQ ID NO:1), and the oligonucleotide (5'-ACAGGTACCCCATAATAGACTGTGAC-3' antisense) (SEQ ID NO:2) containing the *Kpn*I site, used for ligation with the second env fragment. The second fragment was derived by *Kpn*I and *Bam*HI digests of the pSVIII-env plasmid, and the third fragment was generated by PCR with the synthetic oligonucleotide containing the *Bam*HI site at its 5' end (5'-AACGGATCCTTAGCACTTATCTGGG-3', sense) (SEQ ID NO:3) and the antisense primer (5'-TTGCGCGGCCGCTTATAGCAAAATCCTTTCC-3') (SEQ ID NO:4) containing the TAA stop codon followed by the *Not*I site. The three fragments were ligated into the *Sa*II and *Not*I sites of the pSC11-based vector (a generous gift of Dr. L. Eisenlohr; Thomas Jefferson University, Philadelphia, PA) to generate plasmid pSC-ΔV3. A similar approach was used to generate plasmid with the WT env gene (pSC-WTP) using recombinant clone pIIIB (Hwang, et al, *Science* 253:71-74) kindly provided by Dr. B. Cullen (Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC). Plasmids pSC-ΔV3 and pSC-WTP were used to generate vv-ΔV3 and vv-WTP by homologous recombination as described (Earl et al, 1990, *J Virol.* 64:2448-2451).--